

WHAT IS CLAIMED IS:

1           1. A *Lactobacillus* bacterium comprising an expression cassette, the  
2 expression cassette comprising a promoter operably linked to polynucleotide encoding a  
3 signal sequence and a biologically-active polypeptide, wherein the biologically active  
4 polypeptide is linked to a heterologous carboxyl terminal cell wall targeting region and  
5 wherein the heterologous carboxyl terminal cell wall targeting region comprises in the  
6 following order:

7                 a cell wall associated sequence;  
8                 LPQ(S/A/T)(G/A);and  
9                 a hydrophobic sequence.

1           2. The *Lactobacillus* bacterium of claim 1, wherein the cell wall  
2 associated sequence comprises at least 50 amino acids.

1           3. The *Lactobacillus* bacterium of claim 1, wherein the cell wall  
2 associated sequence comprises at least 200 amino acids.

1           4. The *Lactobacillus* bacterium of claim 1, wherein the heterologous  
2 carboxyl terminal cell wall targeting region further comprises a charged sequence at the  
3 carboxyl terminus of region.

1           5. The *Lactobacillus* bacterium of claim 1, wherein the *Lactobacillus*  
2 bacterium is a vagina-colonizing strain.

1           6. The *Lactobacillus* bacterium of claim 1, wherein the *Lactobacillus*  
2 bacterium is selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.

1           7. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises the amino acid sequence LPQSG.

1           8. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises the amino acid sequence LPQAG.

1           9. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises the amino acid sequence LPQTG.

1                   10.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises the amino acid sequence LPQTA.

1                   11.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises SEQ ID NO:7.

1                   12.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises SEQ ID NO:8.

1                   13.     The *Lactobacillus* bacterium of claim 1, wherein the biologically  
2 active polypeptide is expressed in the cell wall of the bacterium.

1                   14.     The *Lactobacillus* bacterium of claim 1, wherein the biologically-  
2 active polypeptide is between 10 and 600 amino acids.

1                   15.     The *Lactobacillus* bacterium of claim 1, wherein the biologically-  
2 active protein binds to a pathogen when the biologically active protein is contacted with the  
3 pathogen.

1                   16.     The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a  
2 bacterial pathogen.

1                   17.     The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a  
2 fungal pathogen.

1                   18.     The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a  
2 viral pathogen.

1                   19.     The *Lactobacillus* bacterium of claim 18, wherein the viral pathogen is  
2 HIV.

1                   20.     The *Lactobacillus* bacterium of claim 19, wherein the biologically-  
2 active protein is CD4 or an HIV-binding fragment of CD4.

1                   21.     The *Lactobacillus* bacterium of claim 19, wherein the biologically-  
2 active protein is 2D-CD4.

1                   22.     The *Lactobacillus* bacterium of claim 18, wherein the biologically  
2 active protein is cyanovirin-N or a virus-binding fragment of cyanovirin-N.

1                   23.     The *Lactobacillus* bacterium of claim 18, wherein the viral pathogen is  
2 herpes simplex virus.

1                   24.     The *Lactobacillus* bacterium of claim 18, wherein the biologically  
2 active protein is herpes simplex virus entry mediator C (HveC) or a virus-binding fragment of  
3 HveC.

1                   25.     The *Lactobacillus* bacterium of claim 1, wherein the biologically  
2 active polypeptide is released from the *Lactobacillus* bacterium.

1                   26.     The *Lactobacillus* bacterium of claim 4, wherein the biologically  
2 active polypeptide is anchored to the cell wall of the *Lactobacillus* bacterium.

1                   27.     A method of expressing a biologically active polypeptide in the cell  
2 wall of a *Lactobacillus* bacterium, the method comprising  
3                   providing a *Lactobacillus* bacterium comprising an expression cassette, the  
4 expression cassette comprising a promoter operably linked to a polynucleotide encoding a  
5 signal sequence and a biologically-active polypeptide, wherein the biologically active  
6 polypeptide is linked to a heterologous carboxyl terminal cell wall targeting region and  
7 wherein the heterologous carboxyl terminal cell wall targeting region comprises in the  
8 following order:

9                   a cell wall associated sequence;  
10                  LPQ(S/A/T)(G/A);and  
11                  a hydrophobic sequence; and  
12                  culturing the bacterium under conditions to induce expression of the  
13 polypeptide, thereby expressing a biologically active polypeptide in the cell wall of a  
14 *Lactobacillus* bacterium.

1                   28.     The method of claim 27, wherein the cell wall associated sequence  
2 comprises at least 50 amino acids.

1                   29.     The method of claim 27, wherein the cell wall associated sequence  
2 comprises at least 200 amino acids.

1                   30.     The method of claim 27, wherein the heterologous carboxyl terminal  
2     cell wall targeting region further comprises a charged sequence at the carboxyl terminus of  
3     region.

1                   31.     The method of claim 27, wherein the providing step comprises  
2     transferring the expression cassette into the bacterium.

1                   32.     The method of claim 27, wherein the cell wall targeting region  
2     comprises the amino acid sequence LPQSG.

1                   33.     The method of claim 27, wherein the cell wall targeting region  
2     comprises the amino acid sequence LPQAG.

1                   34.     The method of claim 27, wherein the cell wall targeting region  
2     comprises the amino acid sequence LPQTG.

1                   35.     The method of claim 27, wherein the cell wall targeting region  
2     comprises the amino acid sequence LPQTA.

1                   36.     The method of claim 27, wherein the cell wall targeting region  
2     comprises SEQ ID NO:7.

1                   37.     The method of claim 27, wherein the cell wall targeting region  
2     comprises SEQ ID NO:8.

1                   38.     The method of claim 27, wherein the cell wall targeting region  
2     comprises at least 200 amino acids.

1                   39.     The method of claim 27, wherein the *Lactobacillus* bacterium is a  
2     vagina-colonizing strain.

1                   40.     The method of claim 27, wherein the *Lactobacillus* bacterium is  
2     selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.

1                   41.     The method of claim 27, wherein the biologically-active polypeptide is  
2     between 10 and 600 amino acids.

1                  42.     The method of claim 27, wherein the biologically active protein binds  
2 to a pathogen when the biologically active protein is contacted with the pathogen.

1                  43.     The method of claim 42, wherein the pathogen is a bacterial pathogen.

1                  44.     The method of claim 42, wherein the pathogen is a fungal pathogen.

1                  45.     The method of claim 42, wherein the pathogen is a viral pathogen.

1                  46.     The method of claim 45, wherein the viral pathogen is HIV.

1                  47.     The method of claim 46, wherein the biologically active protein is CD4  
2 or an HIV-binding fragment of CD4.

1                  48.     The method of claim 46, wherein the biologically active protein is 2D-  
2 CD4.

1                  49.     The method of claim 45, wherein the biologically active protein is  
2 cyanovirin-N or a virus-binding fragment of cyanovirin-N.

1                  50.     The method of claim 45, wherein the viral pathogen is herpes simplex  
2 virus.

1                  51.     The method of claim 45, wherein the biologically active protein is  
2 herpes simplex virus entry mediator C (HveC) or a virus-binding fragment of HveC.

1                  52.     The method of claim 27, wherein the biologically active polypeptide is  
2 released from the *Lactobacillus* bacterium.

1                  53.     The method of claim 30, wherein the biologically active polypeptide is  
2 anchored in the cell wall of the *Lactobacillus* bacterium.

1                  54.     A method of providing a biologically active protein to a mammalian  
2 mucosal surface, the method comprising,

3                      contacting a mucosal surface with a *Lactobacillus* bacterium recombinantly  
4 altered to express a signal sequence linked to a biologically-active polypeptide linked to a  
5 heterologous carboxyl terminal cell wall targeting region, the heterologous carboxyl terminal  
6 cell wall targeting region comprising in the following order:

7                   a cell wall associated sequence;  
8                   LPQ(S/A/T)(G/A); and  
9                   a hydrophobic sequence,  
10                  wherein the biologically active polypeptide is expressed in an amount able to  
11                  be detected in a sample collected from the mucosal surface.

1                 55.       The method of claim 54, wherein the cell wall associated sequence  
2                  comprises at least 50 amino acids.

1                 56.       The method of claim 54, wherein the cell wall associated sequence  
2                  comprises at least 200 amino acids.

1                 57.       The method of claim 54, wherein the heterologous carboxyl terminal  
2                  cell wall targeting region further comprises a charged sequence at the carboxyl terminus of  
3                  region.

1                 58.       The method of claim 54, wherein the *Lactobacillus* bacterium is  
2                  selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.

1                 59.       The method of claim 54, wherein the mucosal surface resides within  
2                  the vagina.

1                 60.       The method of claim 54, wherein the mucosal surface resides within  
2                  the gastrointestinal tract.

1                 61.       The method of claim 54, wherein the contacting step comprises orally  
2                  administering the *Lactobacillus* bacterium.

1                 62.       The method of claim 54, wherein the contacting step comprises  
2                  vaginally administering the *Lactobacillus* bacterium.

1                 63.       The method of claim 54, wherein the contacting step comprises  
2                  rectally administering the *Lactobacillus* bacterium.

1                 64.       An expression cassette comprising a promoter operably linked to a  
2                  polynucleotide encoding a signal sequence and a biologically-active polypeptide, wherein the  
3                  biologically active polypeptide is linked to SEQ ID NO:7 or SEQ ID NO:8.

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65. A vector comprising the expression cassette of claim 64.